



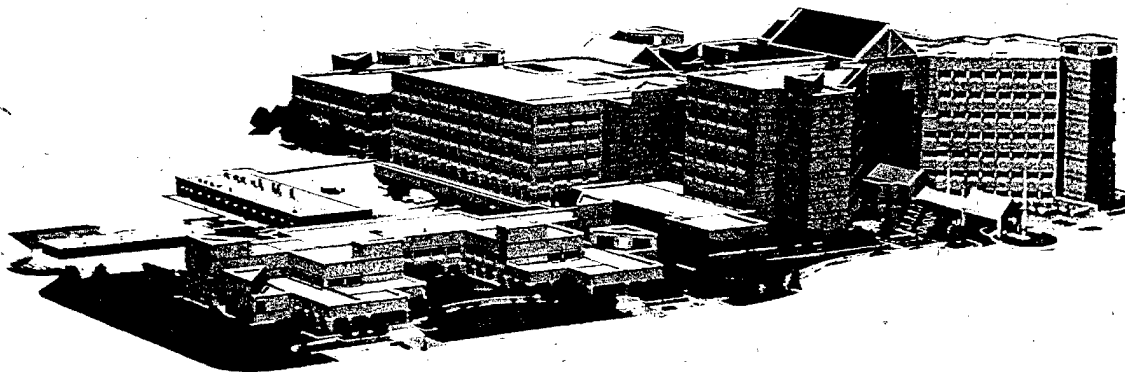
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**The Multiple Inert Gas Elimination Technique:
Current Methodology at the
U.S. Army Institute of Surgical Research**

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USA, MC**

October, 2002



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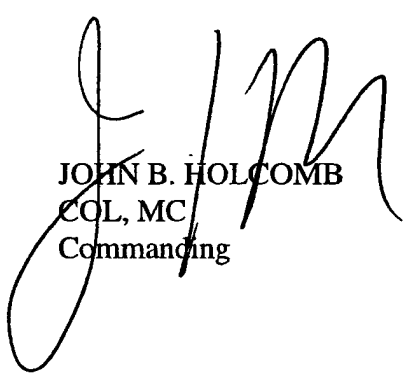
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1. The manuscript entitled "The Multiple Inert Gas Elimination Technique: Current Methodology at the U.S. Army Institute of Surgical Research" by Andriy Batchinsky MD and Lee Cancio MD has been reviewed by this Command and meets acceptable standards for publication as a technical report of the U.S. Army Institute of Surgical Research. The manuscript contains no matter that warrants disapproval for security or policy reasons.
2. The above manuscript has been submitted to the U.S. Army Institute of Surgical Research as a technical report.


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**The Multiple Inert Gas Elimination Technique:
Current Methodology**

At the U.S. Army Institute of Surgical Research

U.S. Army Institute of Surgical Research Technical Report

Number 2002-x

Andriy Batchinsky, M.D., Leopoldo C. Cancio, M.D., LTC, MC, USA

US Army Institute of Surgical Research, San Antonio, TX

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Notes

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

The mention of specific products does not constitute an endorsement.

In conducting the research described in this report, the investigators adhered to the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 85-23. The Animal Care and Use Committee of our Institute approved experimental protocol and animal care.

Abstract

The intrapulmonary causes of hypoxemia include alveolar hypoventilation, ventilation-perfusion (V_A/Q) inequality, shunt, and diffusion limitation to oxygen. In the late 1970s, P.D. Wagner developed the Multiple Inert Gas Elimination Technique (MIGET), a method of assessing V_A/Q inequality (V_A/Q mismatch) and shunt. It can also be used to measure diffusion limitation. In MIGET, the lung is considered as a set of 50 respiratory units, each with a different V_A/Q ratio. Six inert gases of varying solubility are infused, and the arterial, mixed venous, and expired air concentrations of the gases are measured under steady-state conditions. Then, distribution of blood flow and ventilation to each of the 50 compartments is calculated. Although technically challenging, this method can be applied in a wide range of basic and clinical settings, and enables the evaluation of V_A/Q mismatch in a relatively low-risk, non-invasive fashion. This report describes the MIGET methodology from a technical standpoint, as currently performed at this Institute. General set-up; assessment of the gas chromatograph instrument's linearity; preparation and infusion of the 6-gas solution; specimen collection; specimen processing, and data analysis are covered in detail. Creation of a new, complex, multi-ramped GC program is described. Instructional videos form part of the multimedia CD-ROM version of this report.

Introduction

The intrapulmonary causes of hypoxemia include alveolar hypoventilation, ventilation-perfusion (V_A/Q) inequality, shunt, and diffusion limitation to oxygen. In the late 1970s, Peter D. Wagner, MD, of the Division of Physiology of the University of California at San Diego, developed the Multiple Inert Gas Elimination Technique (MIGET)¹⁻¹⁰. This technique provides a quantitative assessment of V_A/Q inequality (V_A/Q mismatch) and shunt. It can also be used to measure diffusion limitation. In MIGET, the lung is considered as a set of 50 respiratory units. Each of these units has a different V_A/Q ratio. Six inert gases of varying solubility are infused, and the arterial, mixed venous, and expired air concentrations of the gases are measured under steady state conditions. Then, distribution of blood flow and ventilation to each of the 50 compartments is calculated and graphically depicted, making it possible to differentiate intrapulmonary causes for hypoxemia.

Shimazu et al. introduced MIGET to this Institute for the study of the smoke inhalation injury in an ovine model¹¹; Dr. Wagner visited in 1987 to assist with technique development. MIGET helped define the primary role of V_A/Q mismatch in the pathophysiology of hypoxemia following inhalation injury¹². In subsequent work, Ogura, Tasaki et al. used MIGET to explore the pathophysiology of sepsis-induced lung injury, and to assess the mechanism of action of various therapies for smoke- and sepsis-induced lung injury in ovine and porcine models¹³⁻¹⁹.

In 2001, Batchinsky et al. reintroduced MIGET to this Institute for the study of pulmonary contusion. New equipment and personnel mandated that all the procedures be relearned. Again, Dr. Wagner visited the Institute to consult during an animal experiment. Subsequently, Dr. Batchinsky visited Dr. Wagner's laboratory and viewed a human experiment. These visits were critical to our success in this effort.

The purpose of this report is to provide a comprehensive description of the MIGET procedures as currently practiced at this Institute. The intent is to enable an investigator who is new to MIGET successfully to implement the technique with minimal assistance. To this end, a multimedia version of this report on CD-ROM has been created, which includes both slides and video clips. Nevertheless, successful implementation of this technique requires dedication, practice, and determination on the part of at least one full-time investigator.

Basic considerations and assumptions

The principle of mass balance

One of the foundations of MIGET is the mass-balance principle⁷. "Mass balance" means that there is conservation of mass. Specifically, the entire mass of a particular inert gas entering the lung from mixed venous blood, exits the lung by both the arterial blood and exhaled alveolar air. This model is represented by the following equation:

$$\lambda P_v Q_T = \lambda P_a Q_T + P_E V_E \quad (1)$$

where P_v is the mixed venous tension of the inert gas, P_a is the arterial tension of the inert gas, P_E is the mixed expired air tension of the inert gas, V_E is the minute ventilation, Q_T is the cardiac output, and λ is the solubility, expressed as the blood-to-gas partition coefficient of the inert gas.

The partition coefficient λ is the ratio of the concentration of the gas in the liquid phase to the concentration of the gas in the gaseous phase ("head space"):

$$\lambda = \frac{(P_B - P_{H_2O})}{100} \beta \quad (2)$$

where β is the solubility of the inert gas in ml, per 100 ml of blood per mmHg (ml/100ml/mmHg); P_B is the barometric pressure in mmHg; and P_{H_2O} is the water vapor pressure (obtainable from standard tables).

For equation (1) to hold true, Henry's law must apply: there is a linear relationship between the partial pressure of the gas and the mass of the gas dissolved in the blood. In addition, the gas is inert; it does not react with the body, and it has a rapid and virtually unlimited diffusion capacity across the alveolar-capillary membrane.

Applying equation (1) to an individual lung unit, which is characterized by a specific V_A/Q ratio:

$$P_c = P_A = P_v * \lambda / (\lambda + V_A/Q) \quad (3)$$

where P_c is the end-capillary partial pressure for the gas and P_A is the alveolar partial pressure for the gas. Thus,

$$P_c / P_v = P_A / P_v = \lambda / (\lambda + V_A/Q) \quad (4)$$

where P_c/P_v defines the retention (R) of the gas in the blood, and P_A/P_v defines the excretion (E) of the gas to the ambient air. From the above we can further rearrange the equation to:

$$R = E = \lambda/(\lambda + V_A/Q) \quad (5)$$

This equation represents an important principle. Namely, the retention and excretion of the inert gas depend only on the solubility of the gas and the V_A/Q ratio of the lung unit. To characterize the various units within the lung the six gases used for MIGET cover a range of partition coefficients from about 0.005 (SF6) to about 300 (acetone). The variety of lung units is represented by application of the equation (5) to each lung unit for each one of the inert gases.

The MIGET data analysis program uses a multi-compartmental approach with enforced smoothing^{1, 3, 7}. Based on the retentions of the six gases, the continuous distribution of the pulmonary blood flow as a function of V_A/Q ratios is estimated. In the same manner, based on the excretion levels of the six gases, the continuous distribution of the alveolar ventilation as a function of the V_A/Q ratios is assessed.

The main variables needed for the program are the partial pressures of the six inert gases in the arterial and mixed venous blood and in the expired air, the partition coefficients for each of the gases, the minute ventilation, and the cardiac output.

The lung

The MIGET does not appreciably alter the physiology of the lung under study. The lung is assumed to consist of a number of compartments (usually 50) that are homogeneous and arranged in parallel. The ventilation and perfusion in every compartment are taken to be constant over a time scale of minutes. The distributions of blood flow and ventilation are modeled as smooth and without sudden irregular changes; there is no inert gas in the inspired air¹.

Steady state conditions and diffusion equilibrium

Another basic assumption is a steady-state condition^{3, 7, 20}. This means that for the validity of the mass-balance equations in all of the V_A/Q units, a steady-state condition must be present to ensure that during sample collection retentions and excretions are constant.

A steady ventilation status can be documented by way of repeated minute-ventilation and respiratory-frequency measurements. In our practice we installed a Wright's respirometer

at the exit port of our mixing box and manually measured the minute ventilation, as the values obtained from the ventilator were often inaccurate.

It is desirable to keep the body temperature of the subject as constant as possible. We attempted to prevent more than 0.5-degree changes in temperature during the infusion. It is also important to note that the "room temperature" which the MIGET software calls for is the actual temperature at the point of ventilation measurements. This temperature should be recorded and entered to the SHORT program. See section "Using the MIGET software".

Steadiness of cardiac output values was assessed by repeated measurements. We determined the cardiac output via thermo-dilution, as an average of quadruplicate measurements for each of the sets of samples taken.

Attention was given to the steadiness of the blood pressure (fluctuations within 10 mm Hg) and of the pulse (within 5 beats/min), as further indicators of a steady state.

Duplicate arterial blood gas samples with similar values would point to a steady state as well.

A good retrospective estimation of steady state during infusion can be obtained from the MIGET data itself. Absence of systematic difference between distributions obtained from the first and second samples is evidence of steady inert-gas exchange. Also, an RSS value in the normal range indicates that the measured data do in fact correspond to a steady-state model of gas exchange.

Characteristics of the inert gases and diffusion limitation

The partial pressure of each inert gas in an alveolus must show a linear relationship to its concentration in the blood passing by the alveolus (Henry's law) ¹. For each of the gases used it is expected that diffusion equilibrium be achieved between alveolar gas and capillary blood in all cases, as the inert gases are invulnerable to diffusion limitation. This principle lays the groundwork for calculations of diffusion limitation, as the arterial PO_2 predicted by the traffic of the inert gases can be compared to the measured PO_2 . If diffusion limitation is present, the PO_2 predicted by the program from V_A/Q inequality and shunt will systematically exceed the measured PO_2 (see VQBOHR program, below).

Selection of subjects for the study

A literature search in the field reveals that MIGET has been performed on a wide variety of subjects: from healthy and diseased humans, through dogs, horses, pigs, sheep, rabbits, lizards and alligators, to avian lungs.

The method has been proven to be safe and widely applicable in clinical settings and in applications in which a steady-state elimination of the inert gas mixture can be assumed. At an infusion rate of 5cc/min, the concentrations of the inert gases in the subject are around 1 part per million and their presence usually goes unnoticed by the subject, except that the taste of ether is noticed by humans under study.

The mechanical ventilator

As long as the expired gas goes directly from the subject to the mixing box, without first passing through the ventilator, the composition of the ventilator should not affect the results. However, the accurate measurement of minute ventilation requires the use of a reliable instrument such as a Wright's respirometer, or, alternatively, a calibrated pneumotachometer. We do not recommend relying on data obtained from the ventilator, with the possible exception of the $F_{I}O_2$.

Supplies

Syringes

Matched plunger and barrel glass syringes should be used for the MIGET.

In our practice we used Becton Dickinson & Co. Yale matched and numbered plunger and barrel syringes of 1, 10, 30, 50 and 100 cc capacity. From the purchased syringes, those of the best quality syringes were selected and their numbers recorded. Syringes must have smooth plunger movement in the barrels, must be tight when closed, and must be able to withstand applied pressure and suction without noticeable leak. Some of the brand-new syringes were completely unusable for the study.

Stopcocks

We used Becton Dickinson 4-way metal stopcocks for making up the MIGET infusate bags and for administering the infusate to the subject.

For all the sampling syringes we used Discofix 4-way stopcocks with luer locks. The comparative advantages of using these stopcocks are: the possibility to lock them to other stopcocks when connecting syringes and/or sampling sites, and low cost. The disadvantage in using these stopcocks is that they are dissolved by high levels of acetone, ether and halothane, which means that they cannot be used in any application in which they would come in direct contact with concentrated forms of those agents (i.e. they can not be used in preparing or delivering the MIGET infusate). For this purpose the more expensive metal stopcocks need to be purchased.

Inert gases

- **SF 6, Ethane and Cyclopropane:** were ordered as a three-gas mixture from Matheson Tri-Gas Inc.
 - Cylinder pressure: 178 psi
 - Cylinder volume: 50 ft³
 - Sulfur Hexafluoride: 20 %
 - Cyclopropane: 20 %
 - Ethane: Balance.
- **Halothane:** (2-bromo-2chloro-1, 1, 1-trifluoroethane) was purchased from Fluka Chemika, 16730, 100 ml.

- **Ethyl Ether:** Fisher certified ACS, anhydrous, E – 138 1, UN 1155, provided in 1-liter metal bottles or Fisher, Ethyl Ether certified spectranalysed, class 1 A, 1 Liter, E 197-1, UN1155. Stored in aluminum bottles.
- **Acetone:** Fisher certified ACS, Class 1 B, A- 18 500, UN 1090 or Fluka Chemika, 00568, 1 liter.
- **Ethrane:** Enflurane, USP obtained from Baxter Healthcare Corporation. Ethrane can be used instead of Halothane.

Heparin

In our studies we used heparin derived from beef lung (Heparin Sodium Injection USP, 1000 units /ml, Pharmacia & Upjohn)²¹. Any type of heparin can be tested for acetone contamination via GC, and it is advisable to test every lot to be used (by obtaining a head space sample and putting it thorough the GC). The question boils down to the magnitude of acetone contamination and its possible influence on the experimental error. In practice the amounts of acetone used in the MIGET are huge compared to any possible acetone contamination of the heparin. In addition, the usual volume of heparin in a single sampling syringe fluctuates between 0.5 and 0.8 gram (depending on brand of the syringe and technique of heparinization, see "Heparinization" below) and is diluted by 7 cc (in our case) or more of blood. We have also successfully used porcine intestine-derived heparin Elkins Sinn Inc. Heparin sodium injection 1000 units/ml (derived from) before we purchased beef lung derived heparin.

Water bath

A Lindberg Blue M water bath (model # RSWB 3222A), equipped with a metal tray, was used. The temperature was always set at 38 degrees C for all the solubility and time-point samples. (It is not necessary to set the water bath temperature to the temperature of the subject.) The agitation was usually between 3 and 4. According to Dr. Wagner, in general, a shaking rate of approximately a 100/min should be applied.

Scale

A Sartorius GMBH digital scale (type 1212 MP) was used for all weighing done in the study. Syringes (empty and filled with blood) are weighed in grams to 2 decimal places

Testing the GC for linearity

See instructional video

Testing the GC for linearity is essential before undertaking MIGET studies⁸. Absolute calibration of the system, however, is completely unnecessary: calculations are done with ratios of inert gases in the expired, arterial and mixed venous samples. When analyzing data, ratios of peak heights, or areas under the peaks determined by the integrator, work equally well. Linearity could be checked with respect to either or both of these variables. We used peak heights in our studies. If no sophisticated integrator is available then manual measurements of peak heights, using a ruler, might work as well.

To perform a linearity test you will need four 30-cc syringes: 1 for the 3-gas mixture of SF₆, ether, and cyclopropane, and 1 for each of the other gases (halothane, ether, acetone).

1. Take a syringe prepared as usual for the expired air sampling and mark it "3-gas".
2. Take 30 cc of gas from the tank and, regulating the outflow by your finger (see instructional video), expel most of the gas from the syringe leaving 3 cc inside.
3. Dilute this amount with nitrogen by taking in 27 cc of it so that the total volume will be 30 cc (3 cc of 3-gas and 27 cc of N₂ or a 1:10 dilution).
4. Expel gas from syringe till 3 cc remain. Repeat step 3 three more times reaching the concentration of 1:10 000. This will be your starting point concentration for the linearity study.
5. Transfer the mixture to a clean syringe.
6. Analyze 5 cc of the 1:10 000 mixture via GC.
7. From here you can do serial dilutions by 1/3 (expel remaining gas from the syringe to leave 20 cc in it and take in 10 cc of N₂. Put 5 cc through the GC and repeat step 6 again. Do this repeatedly reaching the desired amount of dilutions or by 1/2 (expel gas from the syringe to leave 15 cc in it and take in 15 cc of N₂, etc.).
8. Repeating step 7 you can build a sequential dilution linearity graph where Y is the relative concentration and X the peak height (or area).
9. Usually 6-8 serial dilutions are adequate.

10. Repeat all steps for each of the gases. Use headspace gaseous samples from the bottles with liquids (halothane or enflurane, ether and acetone). To obtain a headspace sample take a clean 30 cc syringe and aspirate some amount from the space above the liquid in each bottle. When performing this close the opening of the bottle from which you sample with your palm. This is your headspace sample to use for further dilutions.

General remarks: GC linearity can be verified via the above method as many times as desired. In practice, however, there is no need for reevaluations during a study if all the samples and runs are repeatable and reasonable, and GC operating conditions and hardware remain unchanged.

Gas chromatography

The following describes our setup, but any functional GC with ECD and FID detectors will work with a variety of columns under a variety of conditions that should be optimized for each system.

See Power Point presentation on GC software usage

General Description

A Hewlett Packard model 6890 equipped with a capillary column (J&W GS-Gas pro SN # 0752927, nominal length 30.0 m, nominal diameter 320.0 micrometers) was used for the study. The appropriate acquisition methods for the study were saved in files named PULMCONT.M and Haloth1.M (both identical). These methods contain all the GC settings, and data acquisition files necessary for qualitative and quantitative assessment. Below, a summarized setting description is given. It is available for printout from the GC menu "Method".

Back inlet

- Mode: split
- Initial temperature: 250 degrees C.
- Pressure: 38.14 psi
- Split ratio: 8:1. This parameter is critical in adjusting the signal to noise ratio. In our experience due to the small diameter of the capillary column the lower limit for the split ratio was 7:1.
- Split flow: 115.7 ml/min
- Total flow: 133.0 ml/min
- Gas saver was off.
- Gas type: Nitrogen (Ultra-high-purity, Matheson Tri-Gas, Inc. We used constant flow mode at 14.0 ml/min. Nominal initial pressure was 37.36 psi, average velocity 129 cm/sec. The back inlet was used and the column was split.

Oven

The oven operated in the range of 60 to 250 degrees C. The maximal temperature for this oven was 260 degrees C with a 0.05 set equilibration time. A multi staged ramped oven temperature program was constructed for the study:

- The initial set point was 60 degrees C with 1.20 min. hold.
- At 45 C per minute the temperature was ramped up to 175 C with no hold.

- Further at 40 C/ min ramp to 200 C, no hold; and finally at 30 C/ min ramp to 220 C with a 2 min hold. The total length of each run was 7.06 minutes.
- It took approximately 3 minutes for the oven to cool down between runs. I.E. the overall time per sample was 10 minutes.

When constructing programs for the GC different approaches and settings could be found equally feasible. The finding of each particular regimen is a process of trial and error. Theoretically, using a wider diameter and shorter length column and an isothermal program would be most beneficial from the point of time efficiency as quicker run times could be achieved and no cooling time would be required between runs. In our particular case we adopted the above-mentioned settings, taking into account both the physical properties of our capillary column and concerns about the long-term performance of the equipment.

Detectors

Front detector micro ECD: SF 6 detection

- Temperature: 300 C
- Mode: constant makeup flow.
- Makeup flow: 45 ml/min, Nitrogen.
- Electrometer: on

Back detector FID: ethane, cyclopropane, halothane (ethrane), ether, and acetone detection

- Temperature: 250 C
- Hydrogen flow: 30.0 ml/min
- Air flow: 400.0 ml/min
- Mode: constant makeup.
- Makeup flow: 30.0 ml/min
- Makeup gas type: Helium.
- Flame: on
- Electrometer: on
- Lit offset: 5.0

Signals

Signal 1 ECD

- Data rate 20 Hz
- Type: front detector-column compensation 1
- Save data: on
- Zero: off

- Fast peaks: off
- Attenuation: 0

Column compensation 1

- Derive from front detector.

Signal 2 FID

- Data rate 20 Hz
- Type: back detector-column compensation 2
- Save data: on
- Zero: off
- Range: 0
- Fast peaks: off
- Attenuation: 0

Column compensation 2

- Derive from back detector.

Post run

Post run time 0.00

Data integration

Data integration parameters are automatically saved into the method files and the program will always try to integrate with the saved algorithm unless specified differently. I.E. the method PULMCONT.M has linked acquisition and integration files saved as part of the method and they will always pop up on the screen via double clicking on the "Data Analysis" off line menu. In any case particular integration sequences can be constructed upon specific demand following the instructions from the operational manuals provided with the 6890 system.

Preparation of sampling syringes

See instructional video

Preparation of the arterial and mixed venous sampling syringes

1. Take a clean and dry 30 cc syringe, and connect a stopcock to it. Mark it "A 1".
2. Weigh and record as "dry weight". All weights to be recorded to 2 decimal places.
3. Take a vial of heparin and, using a sterile needle, aspirate the whole contents of the vial into the syringe.
4. Disconnect needle from stopcock.
5. Holding the syringe vertically, move plunger up and down with rotation several times until heparin lubricates all surfaces that could potentially come into contact with blood during sampling. As a rule we always did this step reproducibly by lubricating the syringe till the "30 cc" mark.
6. During your last plunger movement go to "30 cc" and slowly go up until all the heparin pools in the upper portion of the syringe and **all the gas bubbles** are expressed from the syringe. Keep pushing the plunger upwards slowly until the heparin fills the stopcock. This part may require a little tapping on the upper portion of the syringe and stopcock to make sure the system is anaerobic.
7. Close stopcock.
8. Use another similarly prepared and labeled "A 2" syringe (see steps 1 and 2) to connect and lock to the side port of A 1.
9. Hold both syringes in your left hand and close the stopcocks free ports by your fingers (See instructional video section "Syringe preparation").
10. Open A 1 stopcock to the side port where the A 2 is connected (your fingers closing the third, unoccupied port) and transfer heparin from A1 to A2 making sure all of it is expelled under some pressure and the A 1 stopcock is closed before any air could get into A1.
11. Release your finger from the third port of A 1 and suck in the residual heparin from the dead space of the interconnected stopcocks to A 2.

12. Close stopcock A 2.
13. Dry stopcock A 1 in the stream of air and record heparinized weight.
14. Subtract heparinized weight from dry weight to record the pure heparin weight.
15. Now you will have three numbers: dry weight, heparinized weight and heparin weight. Using the same syringes (keeping track of them by the serial numbers) for blood sampling over and over and comparing these three numbers at start of each experiment will give you an idea about your reproducibility and accuracy.
16. Repeat steps from 5 and further for any amount of syringes (prepared as in steps 1 and 2 and marked accordingly) desired to utilize in the study for either arterial or mixed venous sampling.
17. The heparin left in the last syringe could be used to heparinize the 1 cc plastic disposable syringes that are used for arterial and venous blood gas sampling done simultaneously with MIGET sampling. The 1 cc syringes should be equipped with stopcocks (we reused the same stopcocks used previously for MIGET sampling) and heparinized using the same principle as the MIGET sampling syringes making sure no air is left in the syringes as that will interfere with proper and accurate blood gas analysis.
18. The heparinized 1 cc syringes are marked correspondingly to the MIGET syringes so that later they could be analyzed in the blood gas machine and the results will not get mixed up. We usually luer-locked the 1cc syringes to the side port of the MIGET syringes and stored this manifold till sampling. See instructional video section "Syringe preparation".

Comments

In our study we routinely prepared A1 and A2 for morning sampling and A3 and A4 for the end study sampling. This allowed us to perform duplicate sampling per each time point. It is suggested that until good technique is established one would perform quadruplicate sampling to account for possible loss of samples during processing. By the end of this sample preparation stage the spreadsheet should contain: serial numbers, dry weights, heparinized weights and heparin weights for all sampling syringes. The repeatability of these numbers for the same syringes used over and over is an indication of good handling technique and can screen out occasional rough errors.

Preparation of the expired air sampling syringes

We used 30 cc glass syringes in duplicate, marked in correspondence to the arterial MIGET sampling syringes. Maintenance of these syringes is easy. After each expired air sample was run through the GC, the remaining expired air was expelled from the syringe and the syringe stored with its stopcock closed until further sampling. The only concern with these syringes is to store them apart from highly concentrated ingredients (inert gases), to avoid contamination with overwhelming concentrations of the inert gases. There is no need to clean them or store in any specific way unless they would become visibly unusable. Evidence of a problem would include:

- wet areas inside
- unreasonable results--different from the usually observed, reproducible inert gas results; and/or different from the results obtained from duplicate samples
- stickiness during plunger movements
- cracked stopcocks and other situations evaluated by common sense

When this happens cleaning with detergent, thorough rinsing and drying needs to be done. Also, the technique of mixed expired air sampling itself, i.e. repeated flushing of the syringe with air from the sampling box prior to taking the actual expired air sample, enables correct sample collection.

GC sampling syringes

Each of the blood sampling syringes should have a correspondingly marked 30-cc syringe that will be pre-filled with nitrogen to a certain volume. In our practice we always marked them A 1 GC etc. and filled with them with 15 cc of nitrogen. This serves to flush the dead space of the stopcocks before headspace sample collection from the blood-containing syringes (see section and instructional video part "Sample processing"). The other reason for always putting 15 cc of nitrogen in these "unused" syringes was that it allowed their easy differentiation from those syringes, which already contain headspace samples (usually 8-10 cc volume) as well as from those containing expired air samples (30 cc volume). This simple adjustment was useful in situations when a lot of samples were being processed and mistakes might have occurred.

Nitrogen reservoir syringes

We always had two 50-cc syringes filled with nitrogen and marked N 2. These syringes were used to add nitrogen to the blood sample containing syringes. (See "Sample processing").

Preparation of the six-gas infusate

See instructional video

1. Take a bag of 5% Dextrose.
2. Through the rubber aspiration port of the bag introduce an 18-gauge metal spinal needle with a metal or polyvinyl chloride stopcock attached to it.
3. Lay the bag on the table and push all the air together into a big bubble in the middle under the upper cupola of the bag. Open the stopcock and, while applying constant pressure, let the air out. If needed, tap on the bag and remove the remainder of the air in a second maneuver.
4. Hang the bag from an IV pole and connect the hose from the tank containing SF₆, Ethane and Cyclopropane to the stopcock. Open vent and let the gas flush the hose and stopcock for some time (seconds) to make sure you will avoid putting air into the bag.
5. Open the stopcock and fill the bag until it is somewhat distended. Our practice was to fill the bag with gas until the level of fluid was displaced till the mark of 3 (i.e. 300 cc).
6. Close the stopcock and shake the bag for 10-15 seconds. Do not penetrate the bag with the needle. We used a blunt ended needle and penetrated the stopper with a disposable needle prior to inserting the blunt ended needle.
7. Put the bag on the table for 5 min to allow all the small gas bubbles to coalesce. You can skip the 5 minute delay if you facilitate coalescing by tapping—however, keep in mind that insoluble gases are easily driven out of the solution by agitation.
8. Repeating step 3, remove all gas from the bag.
9. Repeat steps 5,6,7,8 two more times. It is sufficient to saturate the fluid once however repeated saturations should serve as protection from any technical mistakes. When the saturation of the bag by the 3-gas mixture is complete and the last expelling of gas from the bag is done you are ready for the next step.
10. Take a 3 cc plastic disposable syringe with a wide gage needle (#21 or 22) and take 1.5 cc from the Diethyl ether bottle. Expel some of the chemical to get rid of the air and fill in the dead space with ether. Disconnect needle and inject the ether

to the bag directly connecting the syringe to the stopcock (replace the plastic stopcock for a metal stopcock if metal was not used initially as the liquids dissolve plastic). Aspirate back some fluid to the syringe and flush back to the bag. The amount of ether delivered should be 1-1.2 cc.

11. Using an above described technique inject 10-12 cc of Acetone to the bag.
12. Finally following the same technique, inject 3 cc of Halothane into the bag.
13. Use the last syringe attached to the bag to remove small gas/air bubbles from the bag.
14. The infusate should be marked and dated and is ready to be infused.

Comments

When the bag is prepared, do not shake it heavily. Invert it systematically over time (including during infusion) to facilitate the distribution of halothane, which tends to pool in dependent portions due to its heaviness.

The infusate loses gases over time, so it should be used up within the next 3-4 hours or so. If another infusion is required by the study design, then either the bag should be "refreshed" by adding the components or another bag can be prepared. If one chooses to upgrade the chemicals in the bag the gas levels should be tested experimentally by means of GC so that a standardized and repeatable approach can be insured. In our experience we prepared a "fresh" bag each time because the infusion time points were 5-6 hours apart. However, there is no particular need to know or standardize the infusate gas concentrations, beyond assuring that an amount of agents sufficient for the completion of the experiment is present in the bag.

The infusate can be prepared in Ringer's lactate, dextrose, or normal saline according to availability and/or study design.

It is not necessary to check the amounts of the components in the prepared infusate bag. However if someone would decide to do so, checking the reproducibility and quality of bag preparation is easily accomplished by taking a 1-cc sample of liquid from the prepared infusate bag and injecting 0.25 cc of it into a container (like a 1 liter plastic Tedlar bag pre-filled with nitrogen) and incubating it for some time (10-15 min) on top of the GC. Then take a clean GC sampling syringe and take some gas phase sample from the bag and run it through the GC. If desired, such internal calibration could be done in the beginning. After some experience with the MIGET each and any of the samples obtained from the subjects (be it expired air or blood sample) could serve as an "internal standard," as with good quality technique the results are highly reproducible.

Collection of blank blood samples for solubility measurements

Before any inert gas infusion was started, it was our routine to collect 7 cc of arterial and venous blood into pre-weighed and pre-heparinized syringes marked A con and V con respectively (see "Solubility Calculations"). As blanks, these samples served as a control point before each experiment to screen out any spurious signals that could interfere with later analysis.

These samples were processed just like routinely collected arterial samples (see solubility measurements steps 1-8) through one cycle of water bath time, head space analysis and GC acquisition (see sample processing) except we did not expect to find any unusual signals and certainly none in the retention time range of the inert gases. After obtaining the blank head space samples the remaining gas (nitrogen) was expelled from the syringes, the blood was retained in the syringes, the syringes were dried and the dry weight was recorded for A con and V con for the further solubility measurements. For the next steps see the following section "Solubility Measurements".

Solubility measurements

See instructional video

Solubility of the inert gases in blood is dependent on temperature, lipid, protein and hematocrit values and thus is a changing variable. As shown by Wagner et al ², the correlation coefficient (r^2) between dog blood lipid levels and solubility was 0.82 for halothane and 0.70 for SF₆. The correlation coefficients for cholesterol were 0.90 and 0.76 for halothane and SF₆ solubility, respectively. For the phospholipids, the correlation coefficients for the same gases respectively were 0.63 and 0.62. For these reasons, the solubilities for the inert gases should be measured in every subject individually on each experimental day, and these values should be entered into the SHORT program (see Using the MIGET software).

1. Weigh two 30-cc glass syringes (weight including labels and stopcocks) and label them "A con " and "V con".
2. Heparinize them. No need to re-weigh them however.
3. Collect about 7 cc of arterial and venous blood correspondingly in to each of the syringes before starting inert gas infusion.
4. Inert gas infusion now may be started as desired.
5. Add 10 cc of nitrogen to each syringe and equilibrate in water bath for 40 minutes.
6. Transfer gas phase to prepared and correspondingly labeled GC sampling syringes and run samples through the GC. This step is done routinely to make sure there are no contaminants in the blood that may interfere with measurements. No weighing /volume recording needed here. After transferring the gas phase to the GC sampling syringes all the residual gas should be thoroughly expelled from the syringe and the syringe and stopcock meticulously dried out before next step.
7. Add 15 cc of the 6-gas mixture described below to each of the blood containing syringes (A con and V con).

To prepare the mixture you need five 50-cc glass syringes. (The dilutions can be done with room air if there is no N₂ available. However, the air should be free of any substances being analyzed.)

- **Syringe # 1:** label it " 3 GAS" and fill it up with gas from the tank containing SF₆, Ethane and Cyclopropane. Dilute this gas by 4 successive lots of N₂ (5 cc of 3 gas plus 45 cc of N₂ each time). Lay it down on the table.
- **Syringe # 2:** label it "Halothane" (or "Enflurane" depending on which one is used) and fill the syringe with headspace from the halothane bottle, after which dilute it by 2 successive lots of N₂ (two times 5 cc gas plus 45 cc N₂).
- **Syringe # 3:** label it " Diethyl ether " and fill this syringe with headspace from the Diethyl ether bottle diluting it in a ratio of 5 cc headspace gas to 25 cc of N₂.
- **Syringe # 4:** label it " Acetone "and fill this syringe with headspace from acetone bottle. **DO NOT** use any dilutions.
- **Syringe # 5:** label it "6 gas" and combine in it:
 - 15 cc from the "3-gas" syringe
 - 10 cc from the "Halothane" syringe
 - 5 cc from "Diethyl ether" syringe
 - 10 cc from "Acetone" syringe
 Use syringe 5 to add 15 cc of 6-gas mixture to the A con and V con syringes.

1. Equilibrate A con and V con syringes for 40 min in the water bath.
2. Remove syringe A con from the water bath, blow its stopcock dry with air from a tank or wall supply and transfer gas phase to a GC sampling syringe labeled S1. S1 is a 30 cc dry and clean glass syringe previously filled with 10 cc of N₂. The nitrogen will be expelled from the S1 syringe after A con and S1 syringes are connected by the stopcocks and just prior to the headspace sample being transferred from A con to S1. See section "Preparation of sampling syringes," subsection "GC Sampling Syringes". **Important: avoid any moisture in the stopcocks while transferring the sample.** With good skills this transfer can be accomplished in 10-15 seconds.
3. Disconnect A con and S1.
4. Put S 1 on the table and **quickly express all residual gas (including tiny bubbles) from the A con syringe to ensure full first extraction of inert gases and avoid re equilibration of the gases with the blood remaining in the syringe.** Leave about 5 cc of blood in A con.
5. Repeat steps 2, 3 and 4 for V con. The sampling GC syringe should be marked S 2 for the gas phase V con sample.

6. Run S1 and S2 through the GC.
7. Dry and weigh A con and V con syringes. Compute blood volumes from weights using weight of blood and blood density (measured later from pooled blood in a graduated cylinder). Call net blood volumes VB1 and VB2 respectively.
8. Add 15 cc of N_2 to the A con and V con.
9. Equilibrate for 40 min. in water bath. During the water bath period make sure the plunger rotates freely in the syringe and is not stuck.
10. 5 minutes before the end of the 40 min water bath period stop the water bath shaker and accurately read the total volume of blood and gas in the syringe. Turn up the shaker and finish the 40 min incubation.
11. Remove syringe A con from the water bath, blow its stopcock dry and quickly transfer the gas phase to a 30-cc glass GC sampling syringe labeled S 1 R. This syringe should be prepared similarly to S1 described above.
12. **Important: avoid any moisture in the stopcocks while transferring the sample.** With good skills this transfer can be accomplished in 10-15 seconds.
13. Put S 1 R on the table and **quickly express all residual gas (including tiny bubbles) from the A con syringe to ensure full first extraction of inert gases and avoid re equilibration of the gases with the blood remaining in the syringe.**
14. Repeat steps 11, 12 and 13 for V con. The sampling GC syringe should be marked S 2 R for the gas phase V con sample and prepared similarly to S1, S2 and S 1 R.
15. Call net gas volumes (total volume minus VB1 or VB2) VG1 and VG2 respectively.
16. Run samples S 1 R and S 2 R through the GC.
17. Next steps are only for acetone and diethyl ether solubilities. For this you need two more 50-cc glass syringes with 3-port stopcocks, one 1-cc glass tuberculin syringe and an 18-gauge spinal (i.e. long-shaft metal needle). Fill the 50-cc syringes with exactly 40 cc of N_2 and mark them accordingly (we mark S 1 R 1:50 and S 2 R 1:50). Fill the 1-cc syringe with blood from the A con syringe. This takes an initial priming/expulsion (1,2,3 times of necessity) to make

sure there are no bubbles in the syringe after a final draw of blood. Do not get air into A con during this transfer. Connect the 1-cc syringe to the side port of the 3-port stopcock and the 18-gauge needle to the third port. When priming/expulsion, turn off/shut off the port with the needle attached to it and draw 1 cc of blood from the A con. Then shut off the A con port and push out the blood from the 1-cc syringe, holding the 1 cc vertically and tapping it slightly to ensure expelling of gas bubbles. Repeat cycle until a gas free sample of blood in the 1-cc syringe. **(The availability of blood in the A con is usually the limiting factor for iterations in this step. In our practice 2-3 priming expulsions were sufficient and the 4-5 cc of blood left in the A con and V con after the equilibrations was always plenty for this purpose).** When a gas free sample of blood is available in the 1-cc syringe we expel some blood out through the needle (A con port shut off) leaving 0.25 cc of blood in the tuberculin syringe (use the volumetric mark in the tuberculin syringe). **The dead space of the needle should be filled with blood from the 1 cc syringe.** (That is why you need about 1 cc of blood in the tuberculin syringe i.e. to have enough blood to leave 0.25 cc in the syringe and flush/occupy the dead space with gas free blood). The whole manifold is taken to the S1R 1:50 syringe.

18. **Quickly:** open the stopcock of the S1R 1:50, insert the metal needle to approx. 1/3 of the length of the 50-cc syringe barrel and **CAREFULLY**, via rotational movements of the 1-cc syringe plunger, expel the 0.25 cc of blood into the 50-cc syringe **STRICTLY AVOIDING** any blood from getting on the plunger of the 50-cc syringe. When blood is in the S1R 1:50 **IMMEDIATELY** withdraw the needle from the syringe and close its stopcock. **As you expel the 0.25 cc to the 50-cc syringe make sure it lays horizontally on the table and the plunger will not be touched during the whole maneuver that will change the 40 cc volume of N₂ in the syringe and/or suck in air into the syringe.**
19. When you are done with this keep the S 1 R 1:50 syringe at a 45-degree angle (avoiding blood contamination of the plunger) and place it to the water bath for 5 minutes with slight shaking **keeping it at 45 degrees.** We routinely performed this by holding the syringe inside the water bath by hand. The main concerns here are letting the plunger move/expand freely (as the inert gases come out of the blood) and, also, preventing water of getting between the plunger and barrel of the 50-cc glass syringe.
20. After 5 minutes of incubation read and record total volume in the syringe (the volume gain is usually in order of 3- to 5-cc plus to the 40-cc initially in the syringe), blow stopcock dry and quickly transfer the gas sample to a previously prepared GC sampling syringe which we usually mark correspondingly (S 1 R 1:50). **When transferring keep the 50 cc syringe at 45 degrees and the GC sampling syringe horizontally.** Call the net gas volumes (total volume minus

0.25 cc) VG1 and VG2 respectively. Call the blood volumes VB1* and VB2*-- they are of course 0.25 ml each. Run S1R 1:50 through the GC.

21. Repeat all for V con (S2R 1:50).

Calculations

For all data coming from syringe A con, SOL 1 is the blood-gas partition coefficient for any gas, and peak heights from S1, S1R, and S1R 1:50 samples are called P1, P1R and P1R * respectively,

$$\text{SOL1} = (\text{VG1} / \text{VB1}) / (\text{P1} / \text{P1R} - 1)$$

for SF6, ethane, cyclopropane and halothane, and

$$\text{SOL1} = (\text{VG1}^* / \text{VB1}^*) / (\text{P1} / \text{P1R}^* - 1)$$

for diethyl ether and acetone.

Identical calculations for SOL2 come from S2, S2R and S2R* using sample V con.

Comments

The timing of all steps is adjusted so that there is minimal delay between obtaining a GC sample and analyzing it with the GC. The reason for this is that leaking of samples from the sampling syringes occurs at different rates, but generally was reported to be 5-10 % per hour. According to P.D. Wagner a leak rate of 1-2% per hour is acceptable but a faster leak rate mandates that GC be performed immediately after sample acquisition. In every case this can be established experimentally. Our routine approach was to cut down on delay times making them sometimes 20 minutes but most of the time between 0 and 10 minutes. The blood samples once in the syringes theoretically can be utilized hours later, however, we always used them immediately and that should be the goal.

The five 50-cc syringes can be reused every day. There is no need to wash them on a daily basis. The GC sampling syringes were routinely "washed" by successive flushing with N₂. The quality of this "washing" was checked chromatographically. All the syringes, which came into contact with blood, were hand-washed from blood with a brush, were left soaking in water containing a detergent, were thoroughly rinsed by water, and were autoclaved. When completely dry and clean, they were assembled with new stopcocks before each experiment.

Measurements of blood density

Measurement of blood density is accomplished by pooling the residual blood from all the blood sample syringes to a graduated cylinder or disposable syringe. Subtracting the empty weight of the reservoir (cylinder or syringe) from the total weight of the reservoir with the blood we find the weight of blood. Next we divide the weight by the volume of blood and we get blood density. This number is used to calculate blood volume in the syringes by dividing the weight of the blood by the density.

General set-up for infusion and sample collection

Thirty-kg female swine were used in the study of pulmonary contusion in which we employed MIGET. The femoral artery and vein catheterized on both sides. On the right side we administered the anesthesia medication (ketamine and propofol by continuous infusion) via the femoral vein, and we used the femoral artery catheter for sampling. The MIGET infusate was delivered via the left femoral vein (see below "Infusion").

A heating blanket was available to control core temperature if necessary.

There is a lot of confusion regarding loss of soluble gases to the materials in the ventilators used in studies. **To avoid this phenomenon we connected the ventilator with the aim of excluding it from the MIGET sampling circuit.** This was done as follows: output port of ventilator, inspiratory limb of ventilator tubing, one-way valve, Y connector, endotracheal (ET) tube, Y connector, expiratory limb of ventilator tubing (as short as reasonably possible to minimize absorption of soluble gases by the tubing), input port of the mixing box, exit port of mixing box, Wright's respirometer, input port of ventilator. With this set up the ventilator was completely excluded from the picture.

The ET tube and all the tubing between the ET tube and the mixing box were wrapped with aluminum foil and heated above body temperature to avoid vapor condensation and loss of soluble gases into such vapor.

When sampling, the expired gas was obtained into clean and dry 30-cc glass syringes at the exit port of the mixing box with a certain delay in time in reference to the arterial sampling time. The sum of the volumes of the mixing box and the volume of the expiratory side connecting tubing system was divided by the minute ventilation. That gave us the time delay to account for the mixing of the expired air (exhaled at the time point of the arterial sampling) with the volume of the mixing box. If a sudden irregularity occurs in the rate or volume of ventilation or if the ventilation regimen is changed by the investigator, the sampling should be delayed. At least five minutes of ventilation under the new pattern should be allowed prior to sampling but not less than 3 times the ratio of instrument-box dead space divided by minute ventilation.

The mixing box was at all times heated above body temperature to avoid any vapor condensation and loss of soluble gases.

We have successfully used three types of mixing boxes:

- a 1-liter mixing box with internal baffles that was heated by means of a heating tape which was wrapped around the box. This box is similar to that described by Ferrer et al. 22.
- a 10-liter mixing box with a copper coil (internal diameter 2 inches, length 16 feet fitted into a box). The coil inside the box was heated with a heating tape wrapped around it. This box was graciously lent to us by Dr. Wagner and was the box used for the bulk of the pulmonary contusion study.
- a 4.5 liter box in which a stainless steel coil (internal diameter 1.25 inches, length 228 inches) was heated inside a digital oven. This device was designed by Mr. Guy Drew (see attachment). The coil dimensions were copied from the previous ISR model¹²⁻¹⁹.

Because of its small volume, the 1-liter box with baffles was especially convenient for rapid, repeated sampling. The baffles allowed for complete mixture of expired air inside the box. This was confirmed by demonstrating the absence of respiratory variation in the CO₂ levels measured by capnography at the exit port of the box. The same was done for the other boxes as well. In case of a study in which excessive minute ventilation would occur, a bigger volume box needs to be used.

Infusion

Infusion of the six-gas solution should be administered via a peripheral vein.

It is critical to separate the infusion and sampling sites from each other. At a certain stage of our studies we infused into a femoral vein and sampled from the adjacent femoral artery. This jeopardized the results due to the diffusion of the highly concentrated inert gases from the infusion site to the sampling site through the vessel walls and plastic catheters (the gradient of concentrations is huge!). Infusion into a femoral vein on one side of the subject and sampling from a femoral artery on the opposite side of the body completely resolved the "unexplainable" difficulties we had with the technique at that point.

The time of infusion prior to sample collection is usually 30-45 min. The minimal time required for the inert gases to equilibrate with the tissues to ensure a steady delivery to the subject is 20 min. As a rule we waited 45 minutes after the infusion was started before sampling was performed.

The MIGET bag is taken to the subject:

1. Connect the bag to the infusion line via a male-to-male connector.
2. Set the rate of infusion on the Harvard pump to the desired value (3 cc/min in our case).
3. Set the volume of infusate to be delivered (if necessary).
4. Start infusion and note the time.

Comments

Steady delivery of the infusate into the subject is one of the basic conditions to achieve success with the MIGET technique. To avoid confusion we suggest the following:

- Double-check the delivered amount of the infusate by weighing the bag prior to infusion, in the middle and by the end of the infusion time to verify that the desired amount was delivered (the weight difference should correspond to the delivered amount on the Harvard pump display)
- During infusion, turn the bag upside down several times to ensure that halothane does not pool up in the dependent portion of the bag.

- Keep the rate of infusion in cc/min always at least at half of the minute ventilation rate in l/min (rates of 1/4 to 1/3 of the minute ventilation are used in Dr. Wagner's lab); however signal-to-noise ratio should drive you when selecting the rate of infusion. The latter should be experimentally established in every case.
- During the infusion periodically check plastic connections as with time and repeated use the plastic may develop cracks and cause leakage.
- Periodically check and replace the plastic tubing on the rotating drum of the Harvard pump.

MIGET sample collection

Expired air sampling

At all times collection of samples was performed by the same personnel. Each of them was trained to perform his task.

Expired sampling was done at the sampling port of the mixing box. The sampling port should be located in an area of the box (generally closer to the end of the coil) to ensure collecting samples that are steady with respect to inspiration/expiration revolutions and mixing of the gases in the mixing box. To check this we connected an end-tidal CO₂ probe to the sampling port and if the CO₂ value became a plateau this meant that we were sampling from a steady and suitable site.

The sampling was done in duplicates with a time delay in reference to the arterial sampling. The delay was calculated as follows. When half of the arterial sample was drawn the timer was started and the expired sample (15 cc) was taken smoothly over a 30-sec to 1-min period of time and over several respiratory cycles starting whenever the delay time was up. The delay time equals the sum of the volume of the mixing box and the volume of all connecting tubing, divided by the minute ventilation.

Arterial sampling

Arterial sampling is completed to the prepared sampling manifold (see the video and section on syringe preparation). The manifold consists of the sampling syringe (for example A 1), the heparinized ABG-sampling 1-cc syringe connected to the A1 stopcock side port, and a 10-cc capacity "waste" syringe connected to the side port of the ABG syringe. When the whole system is connected to the stopcock at the sampling site the waste syringe is filled with about 8 cc of blood, and after that the sample is anaerobically taken into A 1 (our usual volume was 7 cc). Next the A 1 stopcock is closed, an extra 1-2 cc of blood is drawn to the waste syringe (to make sure fresh blood will be sampled for ABG), and 1 cc of blood is anaerobically taken into the ABG syringe. After this the ABG stopcock is closed and the blood from the waste syringe is returned to the subject.

Mixed-venous sampling

Mixed venous sampling is done via a Swan-Ganz catheter. The sampling manifold (same as for arterial sampling) is prepared and marked V 1, etc. and is performed involving the same steps (see above "Arterial sampling").

Modalities of MIGET

Originally the technique was described using inert gas levels obtained from expired air, arterial and mixed venous blood via a pulmonary artery catheter. In this case, the cardiac output is calculated by the MIGET software based on the Fick principle. However, the technique can be performed with a similar degree of accuracy and with less invasiveness, using only expired air and arterial inert gas levels. In this case the cardiac output should be measured directly and the value entered to the program ⁶. See Using the MIGET software, below.

In our practice we used both approaches and we think that they are equally usable in situations in which only a few time points are being evaluated, and in which the invasiveness of pulmonary artery catheterization is not a limiting factor. The original technique (involving mixed venous sampling) appeared to be more technically challenging to us, because withdrawing blood from the pulmonary catheter was sometimes difficult to execute as quickly as the arterial sampling. Specifically, low hydrostatic pressures at the PA sampling site limited the withdrawal speed. To address this problem, one needs to apply suction to the barrel of the sampling syringe and, in addition, to drop the extracorporeal portion of the catheter as low as possible to increase hydrostatic pressure and thus to facilitate sampling. Depending on the magnitude of the suction applied, air may be sucked into the sampling syringe around the plunger. According to Dr. Wagner, a small volume of air introduced in this manner, is acceptable and he proceeds with the analysis without attempting to expel it. Meanwhile, one must hold the arterial sampling rate down, to match the pace of venous sampling. One way to facilitate the sampling while avoiding sucking a lot of air into the syringe is to use a plastic syringe as a collecting syringe. Once the blood has been collected it can be injected anaerobically into the glass syringe. Following this logic, performing sampling with smaller size glass sampling syringes would be an option as well.

Finally, with the original modality the overall sample processing time increases and because more samples are required, the error introduced by handling and processing rises as well.

In our opinion the arterial-sampling-only technique is much better suited for cases in which rapid, frequent sampling is done, and in which a lot of samples are being analyzed; however, a reliable way of measuring cardiac output and/or oxygen consumption is required. With this approach the cardiac output should be measured and entered into the program directly. The software estimates the mixed venous levels, based on the arterial and expired levels, the solubilities, and the cardiac output. We were most successful in applying this variant of the MIGET.

A third modality of the MIGET requiring only mixed expired and peripheral venous sampling exists²³. With this approach the inert gas infusate is infused into a peripheral

vein of one arm and the samples are collected from the vein of the opposite arm. This modality is based on the assumption that the tissues of the hand do not metabolize the inert gases and, after 90 minutes of infusion, in a resting position, equilibration between blood and tissues is achieved. In this case the venous blood inert gas levels would reflect the concentrations in the in flowing arterial blood and the obtained values could be substituted for the arterial values in the software. This noninvasive approach is particularly suitable for repeated MIGET studies over several days or even weeks.

MIGET sample processing

The mixed expired samples obtained from the subject are introduced into the GC as soon as possible. It can be proven experimentally that syringes leak with time. The rate of sample loss can be tested via GC if necessary. Our general recommendation is to run the samples with minimal delay, and to pre-test syringes and stopcocks for leaks if longer delays are expected. Leak rates greater than 1-2 %/hour are unacceptable.

Running gaseous phase samples through the GC

See instructional video

The GC is equipped with a constant volume inlet loop with a metal stopcock attached to it. **To inject any of the gaseous phase samples** (for example lets call it Exp. 1):

1. Connect Exp. 1 by the plastic stopcock to the metal stopcock of the injection port.
2. Open Exp. 1 stopcock.
3. Depending on the type of GC set-up, it may be necessary to inject a constant amount of each sample into the GC. We injected 4 cc. The amount of sample injected should not be less than 3 times the volume of the constant volume inlet loop plus the volume of the dead space before the loop. In our case we used a 0.5 cc inlet loop so the minimum amount to inject would be 1.5 cc plus some additional 1 cc. The minimum amount to inject would have been 3 cc.
4. Turn the inlet loop handle down **and leave it down till the end of the run.** By leaving the inlet loop turned down you open the flow of the carrier gas to the inlet loop and doing so all the analyzed sample will get flushed to the column. When turning the handle up immediately after injection you might introduce some variability to your injection technique by not letting the entire sample to be flushed out. If you are going to use the open -close technique make sure you will leave the handle turned down for sufficient time for all the sample to get flushed out from the loop.
5. Put the Exp1 stopcock to "all 4 locked" position and disconnect the syringe from the injection port.

Comments

One can certainly inject any desired amount to the loop. The function of the loop is to introduce an equal amount of sample to the GC. I.e., even if you expel 15 cc of expired air sample only the volume capacity of the inlet loop (0.5 cc in our case) will get introduced to the GC column and the excess gas will exit the loop to the room.

Processing of blood samples

MIGET with arterial sampling only

Previously you had the water bath turned on to reach the desired temperature prior to the samples arriving. We always had the temperature set to 38 degrees C. One can leave this number on any value within the range of the core temperature of the subject in study. Theoretically the body temperature during 6-gas infusion and the water bath temperature should match. **In practice, there is no need to precisely match these temperatures as the MIGET program does correct for differences between body temperature and water bath equilibration temperature via choosing the option "correct for body/bath temperature difference" (see section Using the MIGET software, Step-by-step instructions on using SHORT.exe).**

Lets suppose an A 1 sampling syringe arrived to the lab. From the general syringe preparation done previously you have the dry weight, heparinized weight and heparin weight recorded in the data sheet for each of the sampling syringes.

1. Rinse stopcock and syringe from any residual blood after sampling. We usually did this step right after sampling
2. Thoroughly dry A1 including stopcock.
3. Record dry weight.
4. Connect A 1 and an N 2 reservoir syringe via stopcocks (see video "Sample processing" for all of the following steps).
5. Holding them in your left hand open the N2 syringe stopcock and flush the dead space of the stopcock with about 5 cc of N2 and close the luer lock port of the A 1 stopcock with your finger.
6. Holding A 1 in your left hand with the your finger closing the luer port put the N 2 syringe horizontally at the table and holding the A 1 horizontally as well open A 1 stopcock and draw 8 cc of nitrogen from the N2 syringe into the A 1. Usually for a 7 cc blood sample you would take 8-9 cc of nitrogen. The reason to hold syringes horizontally is to avoid blood from getting into the N2 syringe or, if the

tip of A 1 would be up, drawing too much nitrogen to the syringe due to the plunger movements in any of those directions.

7. Quickly close stopcocks. With good skills this transfer can be done very fast.
8. Put the syringe to the water bath, turn up agitation to approximately 100/min (agitation should be sufficient to cause some blood movement in the syringe but one should avoid foaming) and mark the beginning of the 40-minute equilibration time.
9. During the equilibration time you should gently rotate barrel and plungers of the syringes in the water bath to prevent sticking and to ensure free plunger movement. 2-3 such rotations will do, 10-15 min apart.
10. 5 to 10 minutes prior to the end of the equilibration time stop the shaker. **Read and record** total volume in the syringe i.e. volume of blood plus volume of gas, taking into account the blood line by the tip of the plunger. For example if 8 cc of nitrogen were added to the 7 cc of blood initially in the syringe, then a 15 cc volume would increase to about 16.8 or 17.1 cc after the equilibration. This occurs because gas was added at room temperature, and expanded at 38°C
11. Turn the shaker up and finish the equilibration.
12. Quickly (5 seconds!) remove syringe A 1 from the water bath and blow its stopcock dry quickly (5 seconds).
13. Connect stopcocks and flush all the nitrogen out from the A 1 GC through the luer port of the A 1 and immediately close it with your finger.
14. Hold A 1 vertically with the A 1 GC connected under a right angle quickly open A 1 stopcock and transfer gas phase sample to A 1 GC (see "Syringe Preparation" part "GC sampling syringes". **Important: avoid any blood from being transferred into the A 1 GC. If that would happen transfer the gas sample to another GC sample syringe the same way you did from the A 1 (it is a good idea to always have spare GC sampling syringes ready to use).** With good skills this transfer can be and should be accomplished in 10-15 seconds. The reason to rush is that with cooling down the inert gases could re equilibrate with the blood in A 1.
15. The A1 GC can be introduced to the GC as described above in "Running gaseous phase samples through the GC".

16. Residual blood from A 1 is used to measure blood density for each study separately. See section "MIGET Calculations".
17. **All these steps are repeated for any number and type (arterial or mixed venous) of blood samples being analyzed.**

Comments

Sample processing requires very good syringe handling skills, accuracy and fast pace. In case a large number of samples would be analyzed please note that it is better to leave the drawn blood samples unprocessed for a couple of hours, than to rush with the water-bath phase, obtain gaseous samples and let them sit on the table for more than 30 min. This delay will cause loss of some amount of sample due to leakage from the syringe. With good planning you can achieve minimal delay times between GC runs.

Troubleshooting the loss of soluble gases in the expired air samples

Reviewing the MIGET-related literature, there is not much information devoted to this problem. We have experienced some difficulties with recovery of soluble gases, and would like to share this experience.

The soluble gases may be not recovered from the expired samples in the amounts expected based on the excretion of other inert gases. This may be observed, for example, when water builds up in the expiratory limb of the ventilator circuit. As a rule, the biggest deficit is observed with acetone excretion, as it is the most soluble of all the gases used for MIGET. However, other soluble gases may be lost as well. If acetone excretion is normal and e.g. ether excretion is low, the cause is not a simple wet area in the system; rather, something in the system has affinity for the particular gas lost. To rule out this situation, one should reevaluate the components and connections of the expiratory circuit, track down possible materials that could absorb the gas in question, and replace them with inert materials. Refer to section "Supplies" and make sure you use the right ingredients to avoid this problem.

A classic case of acetone loss was observed by one of us in the lab of Dr. Wagner. The study involved a human vigorously exercising on a bicycle. The subject salivated excessively and the expired air contacted the saliva collected in a container. As a result, all of the late study samples (the later in the study, the more saliva pooled up in the system) showed systematic "insufficiency" of the expired acetone levels. During calculations with the MIGET programs, when no automated correction for expired acetone loss is used, this picture is characterized by low (compared to ethyl ether and halothane levels) excretion values for acetone in the SHORT output file. Reviewing the VQ BOHR output file this problem reveals itself in the distorted shape of the excretion – solubility curve.

The SHORT program allows correction for acetone loss by adjusting its excretion value on the basis of the ethyl ether value. However, if excretion level deficits are accompanied by high experimental errors for acetone and/or other gases, and high residual sum of squares numbers are noted as a result of that, then the simple, SHORT program correction for acetone loss will not improve study results.

Calculations completed on the spreadsheet

Calculations done for the MIGET samples

These are the measurements we obtain during the stages of sample processing (for A1 and any other likewise).

1. A 1 dry weight.
2. A 1 heparinized weight.
3. Weight of heparin found as difference of 2 and 1 above.
4. A 1 weight of blood sample (7 cc). Obtained by subtracting the weight of the heparinized syringe from the weight of the blood-containing syringe. This number divided by blood density gives us the volume of blood in the syringe. Subtracting the volume of blood and volume of heparin (#3) in the syringe from the total volume (see below # 5) we find the volume of gas in the syringe.
5. Total volume of gas and blood. Comes from reading the graduations prior to taking the sample out of the water bath.
6. The volume of blood, volume of gas and volume of heparin obtained here enter the SHORT program calculations.

Solubility calculations

See part solubility measurements. The actual solubility values for each gas are entered into the SHORT program.

Using the MIGET software

The data analysis programs mentioned below, SHORT.exe and VQBOHR.exe, were graciously provided by Dr. Wagner. In addition, Dr. George Cremona (Milan, Italy) graciously provided us with Windows-based software for the entry of the data required by SHORT.exe. During the conduct of this study we transitioned from using Dr. Cremona's software, to using the original DOS version of SHORT.exe for data entry.

Step-by-step instructions on using SHORT.exe

Open SHORT and sequentially follow steps. General rules: **press "Enter" after each step, use capital letters**, take a unified and systematic approach to file names. All the raw data and output files can be viewed with Windows Notepad.

1. Double click on icon to open the program.
2. For the given menu select write output to disk 0, press enter.
3. Next, give a file name like 1025BL3.3 (month, date, baseline sampling, 3.3 is mandatory for the program to work properly. It denotes the extension of the SHORT output file), press enter.
4. Next choose for subject, example (1) data for man (should be used for pigs). Enter.
5. Next, choose 1. You will always want to correct for body/bath temperature differences. Usually the water bath is set to a certain temperature and the program will correct for the difference between the temperature of the water bath equilibration and body temperature of the subject. (See section "Sample processing" with regard to water bath settings.) Press enter.
6. Next, choose 0, press enter. Refer to section "Troubleshooting", "Loss of soluble gases in the expired air samples".
7. Next for usual way choose 0. Enter.
8. Choose 1 for arterial blood. Enter.
9. Choose 0 for HP. Enter.

10. Choose N if raw data are not yet on file, enter. If raw data are on file choose Y and for the next menu enter the name of the file with the data which has the extension 7.7. Enter.
11. Enter a label for data set (any info you want). Enter.
12. Call file name as you called the Short output file (1025BL7.7) except extension is always "7.7" here, enter.
13. For this take the same name as above except the extension is "9.9". This file will be the data file transmitted to the VQ BOHR program, enter.
14. Enter number of data sets (1 or 2 or 16 etc.), P_B (barometric pressure, measured daily in mm Hg with a barometer near the room in which the experiment is conducted), blood-gas electrode temp from the blood-gas machine (37 in our case), the water-bath equilibration temperature (this was 38 C in our case), fractional CV ("correction value" for the gases; for the gases other than SF6 it is 0.03; do not alter this value); and oxygen solubility (in blood) is 0.003, enter.
15. Put in number of gases 6 if all of them used. (One can exclude any of the gases if desired and in that case here the actual number should be entered i.e. 5 if one is excluded etc.). Number of compartments - usually 50, Z smoothing factor usually 40, lowest - 0.005 and highest - 100 compartments. Enter.
16. Enter partition coefficients for the gases used obtained from calculations (see section "Solubility calculations") Example: 0.004, enter, 0.099, enter etc. Should be 6 partition coefficients if 6 gases used.
17. If above entered values correct press Y, enter.
18. Now enter arterial peak height or area (see section "Data analysis" by the GC). After each value press enter i.e. 1200, enter, 25, enter etc.
19. Confirm Y if the data above was entered correctly. Enter.
20. Enter expired peaks in the same way as steps 18-19.
21. Enter actual venous peak values as in steps 18-19 for MIGET with mixed venous sampling; or enter all 0 (zeros) for venous values if you are using MIGET with arterial sampling only. Next, confirm correct choice.
22. Enter: VE-minute ventilation (in l/m), QT cardiac output (in l/m), PB barometric pressure (in mmHg), TEMPB -subjects body temp (in C), TEMPR - temperature

at the point where minute ventilation was measured (at the Wright's respirometer point in our case (in C)), V_GA – volume of the gas in A 1 syringe, V_BA volume of blood in A1, V_HA – volume of heparin in A1, V_GV, V_BV, V_HV all respectively for V1 (all these obtained from MIGET basic measurements sheet). If MIGET with arterial sampling only was performed enter 0,1,0 for the three venous values respectively.

23. Choose Y if above entered is correct. Enter.
24. Enter: hemoglobin, hematocrit, oxygen consumption, CO₂ production, 99000 for tolerance, F_IO₂, F_ICO₂, P₅₀, P_aO₂, P_aCO₂ and pH from the blood gas machine data. **IMPORTANT: the program requires entry of the VO₂ and VCO₂ once again here after the pH value.** Each data value should be followed by pressing the Enter key. We measured hemoglobin from the blood gas machine (using arterial blood); measured hematocrit with a centrifuge; calculated VO₂ from the cardiac output, etc.; calculated VCO₂ = VO₂*0.75; assumed F_ICO₂ = 0; accepted the P₅₀ calculated by the blood-gas machine; and used non-temperature-corrected values for P_aO₂, P_aCO₂ and pH. Note that the program corrects the blood-gas values for temperature. Dr. Wagner provided us with a separate program that calculates the P₅₀ from a series of PO₂ and SO₂ values. However, we did not use this program in the present study.
25. Choose Y if above entered is correct. Enter.
26. You will see the summarized calculations of the SHORT program. Choose Y.
27. When you press enter the screen will disappear and the files 1025BL3.3 (your SHORT output file), 1025BL7.7 and 1025BL9.9 9 (raw data files) will be completed with data.

The files will be automatically saved into the same folder where the SHORT program is located.

Step by step instructions on using VQBOHR.exe

Open VQBOHR and sequentially follow steps. General rules: press "Enter" after each step, use capital letters, take a unified and systematic approach to file names. All the raw data and output files can be viewed with Windows Notepad.

1. Double click on icon VQBOHR to open the program.
2. Into the first menu enter the 9.9 extension raw data file that you created in the SHORT. I.e. 1025BL9.9. Enter.

3. Choose 0. Enter.
4. Create a VQBOHR output file with 33.3 extension. I.e. 1025BL33.3. Enter.
5. Hit 0. Enter.
6. Hit 0. Enter.
7. Choose 0 for usual way. Enter.
8. Press enter. Do not type in anything.
9. Type Y. Enter.
10. If you are evaluating diffusion limitation type in Y. If you are not – N, enter.
11. If you entered N above than the program ends here and a VQBOHR output file is saved to the same folder where the program is located.
12. If you entered Y at step 10 then follow the steps below.
13. Enter the desired number of integration steps within the range. Example 100, enter.
14. Type in 0. Enter. The same VQBOHR output file will have a section of its print out on diffusion limitation in the end of it.

Data interpretation

The short and long output files contain the results of the calculations. The contents of the short output file are self-explanatory. Considering the long output file the main result parameters are as follows:

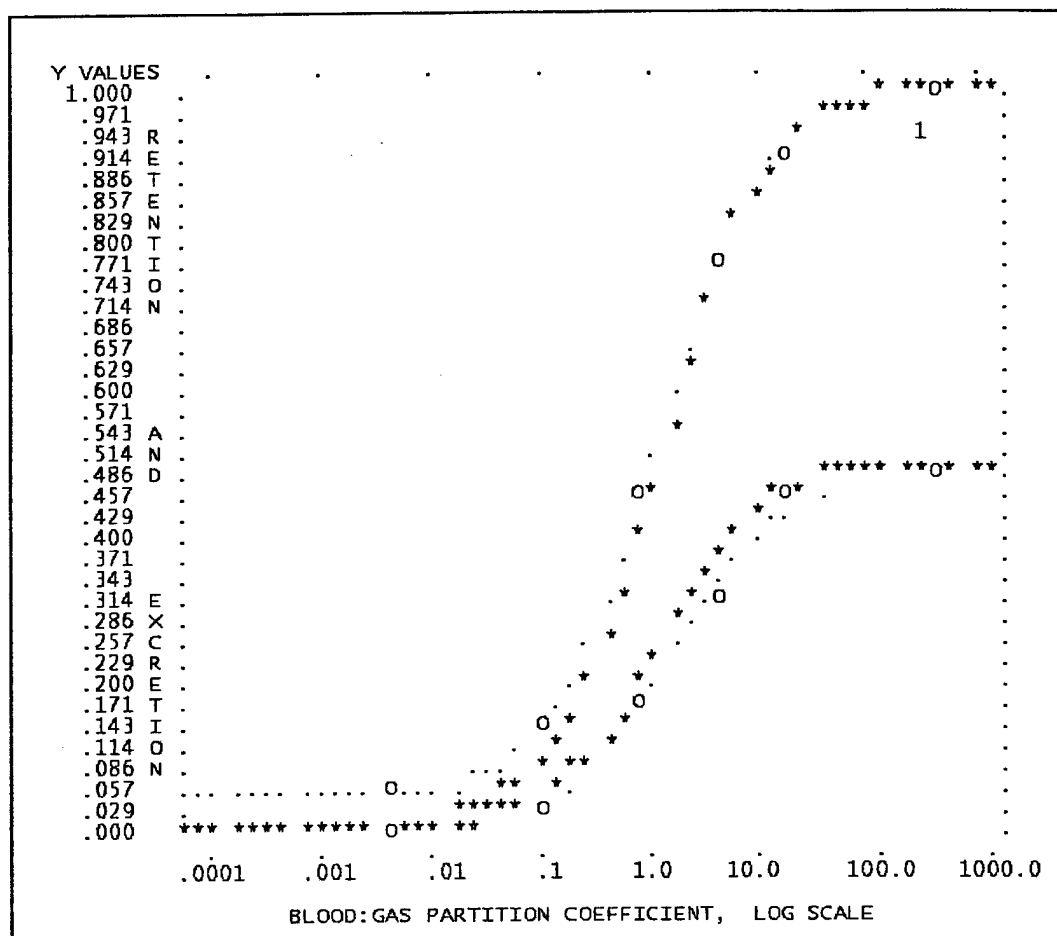


Figure 1. The curves showing the retention and excretion as a function of solubility allow estimation of how close the experimental data came to the expected values for a homogeneous lung. In the depicted case, the fit was good.

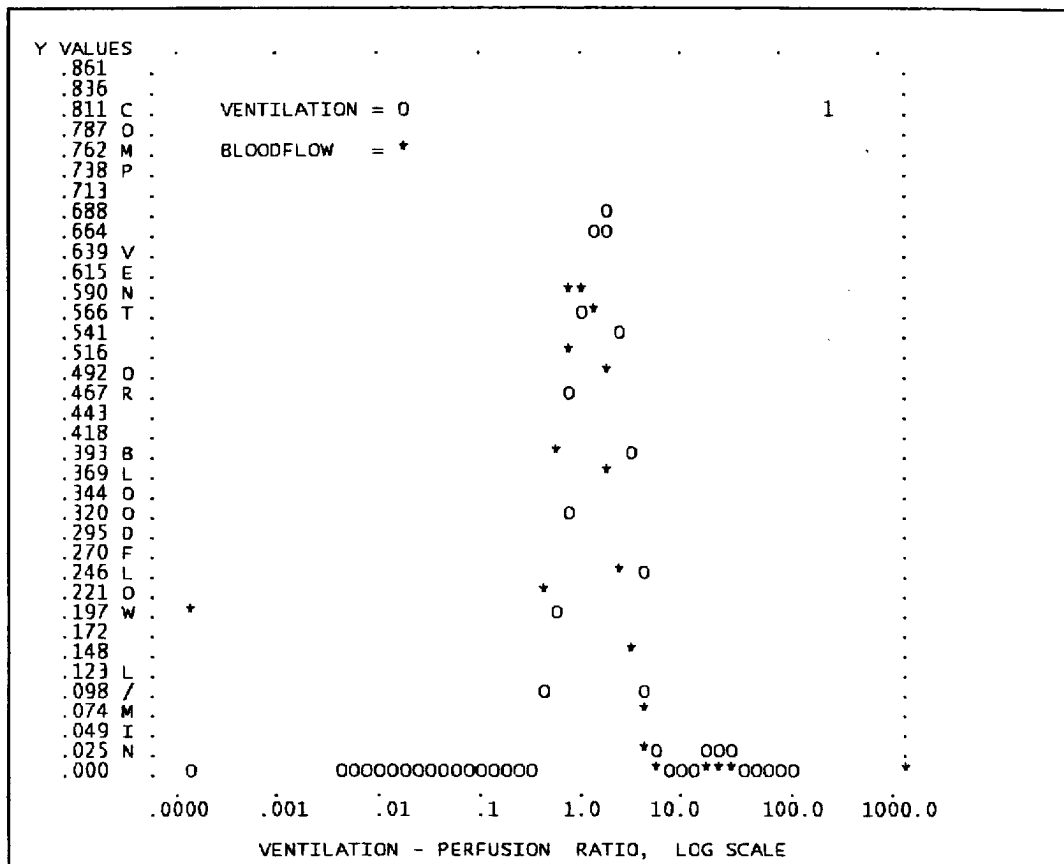


Figure 2. Inspection of the curves allows for a general estimation of the matching between the ventilation and blood flow, as the x-axis represents the V_A/Q ratio and the y-axis the blood flow or the ventilation in liters per minute. A normal or pathologic distribution picture can be inferred from the shape of the curves alone.

PC	RETENTIONS	BEST FIT	ERROR	RAW DATA	ERROR
.0053	8.799	8.533	.266	.05324	-.00161
.1038	17.052	17.449	-.397	.13907	.00324
.7456	29.760	30.428	-.668	.44286	.00994
3.3830	75.125	73.889	1.236	.77930	-.01282
13.9600	221.067	221.594	-.526	.92474	.00220
254.0000	3741.021	3740.952	.068	.99555	-.00002
.0000	20000.000	20000.010	-.010	1.00000	.00000
REMAINING SUM OF SQUARES = 2.48E+00					

Figure 3. The remaining sum of squares (RSS) defines the fit of the data to the model and serves as a quality-control check for the whole set of data. In case of high RSS values, the source of error can be identified by looking at the "Error" column between the "Best Fit" and "Raw Data" columns (see above). That is, if the RSS is consistently high, the cause of this error can sometimes be narrowed down to one or more gases.

As enough data is accumulated (in consecutive experiments in a given laboratory) the remaining sum of squares should behave as follows:

The Chi square expectations on the RSS:

RSS should be < 1.237 2.5% of cases
 RSS should be < 2.204 10% of cases
 RSS should be < 3.828 30% of cases
 RSS should be < 5.348 50% of cases
 RSS should be < 7.231 70% of cases
 RSS should be < 8.558 80% of cases
 RSS should be < 10.645 90% of cases
 RSS should be < 12.592 95% of cases
 RSS should be < 16.812 99% of cases
 RSS should be < 22.458 99.9% of cases

RANGE	BLOOD FLOW	VENTILATION
VA/Q OF ZERO	.046	ZERO
VA/Q RANGE 0 TO .01	.000	.000
VA/Q RANGE .01 TO .1	.000	.000
VA/Q RANGE .1 TO 1.	.520	.161
VA/Q RANGE 1.0 TO 10.	.432	.322
VA/Q RANGE 10. TO 100.	.002	.012
VA/Q OF INFINITY	ZERO	.505

Figure 4. This table provides the fractional distributions of blood flow and ventilation, lumped into arbitrary ranges. In a normal lung the blood flow is fully contained within the range of $V_A/Q = 0.1$ and $V_A/Q = 10$. Abnormally low V_A/Q is < 0.1 and abnormally high is $V_A/Q > 10$. Shunt is specified as blood flow in units with $V_A/Q < 0.005$ and dead space as ventilation in units with $V_A/Q > 100$. The MIGET detects intrapulmonary and intracardiac shunting but is insensitive to shunt resulting from bronchial or thebesian venous admixture.

MEAN OF BLOOD FLOW DISTRIBUTION =	1.03
2nd MOMENT OF BLOOD FLOW DISTRIBUTION =	.53
3rd MOMENT OF BLOOD FLOW DISTRIBUTION =	.07
MEAN OF VENTILATION DISTRIBUTION =	1.44
2nd MOMENT OF VENTILATION DISTRIBUTION =	.66
3rd MOMENT OF VENTILATION DISTRIBUTION =	.34

Figure 5. Several features of these distributions are next provided (see above). The first moment of the distribution (blood flow/ventilation distribution on a log scale) is

the mean abscissa value (V_A/Q) of each curve. The second moment of the distribution (blood flow/ventilation distribution) is the standard deviation for the blood flow and ventilation curves. This indicator is reflective of the standard deviation only if the distribution is logarithmically normal. In case of multi-modal curves the parameter does not necessarily represent the standard deviation but is still a useful parameter of dispersion. The third moment of distribution (blood flow/ventilation distribution) represents the skewness of the curves.

```

***** BOHR INTEGRATION RESULTS *****

ITN 1; DM= 3.17; & PO2 ERROR= -.21
ITN 2; DM= 3.80; & PO2 ERROR= 2.37
ITN 3; DM= 4.76; & PO2 ERROR= 3.92
ITN 4; DM= 3.21; & PO2 ERROR= .06

      ITERATION      PV02      PVC02      +VO2      +VC02
      0              28.62      45.59      .6         18.3

  N    VA/Q    PAO2    PaO2    PACO2    PaCO2    O2CON    CO2CON    RQ
24    .5222    79.41    51.49    40.57    44.06    12.49    53.94    .53
25    .6391    85.47    56.98    39.44    43.46    12.97    53.47    .57
26    .7823    91.71    64.97    38.11    42.70    13.43    52.94    .62
27    .9575    98.12    77.21    36.58    41.74    13.81    52.34    .68
28    1.1720    104.66    93.26    34.82    40.57    14.08    51.67    .76
29    1.4345    111.00    106.63    32.86    39.22    14.22    50.93    .86
30    1.7558    116.69    115.08    30.71    37.76    14.29    50.15    .97
31    2.1491    121.59    120.85    28.42    36.24    14.34    49.32    1.09
32    2.6304    125.76    125.30    26.04    34.70    14.37    48.46    1.22
33    3.2196    129.29    128.93    23.62    33.17    14.40    47.58    1.35
34    3.9408    132.29    131.97    21.21    31.67    14.42    46.71    1.48
35    4.8235    134.83    134.52    18.86    30.24    14.43    45.85    1.61
36    5.9038    136.99    136.67    16.61    28.91    14.45    45.03    1.73
37    7.2262    138.81    138.49    14.50    27.67    14.46    44.25    1.84

MIXED ARTERIAL PN2      =      520.2
MIXED VENOUS PN2       =      557.1
N2 UPTAKE, ML/MIN      =      -1.8

MIXED EXPIRED PO2      =      135.01
MIXED EXPIRED PCO2     =      12.67
OXYGEN UPTAKE          =      159.14
CARBON DIOXIDE OUTPUT  =      152.99
PREDICTED R            =      .96
MEASURED R             =      .85

ARTERIAL PO2           =      66.46
ARTERIAL PCO2          =      38.86

ARTERIAL O2 CONTENT    =      13.55
ARTERIAL CO2 CONTENT   =      50.93
MIXED ALVEOLAR-ARTERIAL PO2 DIFFERENCE =      68.55
MIXED ALVEOLAR-ARTERIAL PCO2 DIFFERENCE =      26.19
MIXED ALVEOLAR-ARTERIAL PN2 DIFFERENCE =      10.50

DL02 by Bohr Integration =      3.21
DLC02 set to 5*DL02     =      16.06
NUMBER OF STEPS USED    =      100

```

Figure 6. The program allows estimation of diffusion limitation for subjects breathing 21% O₂ and below. The F_IO₂ must be .21 or less for this calculation. With

the exception of the example provided below, we used an $F_{I}O_2$ of .50 in the present study; therefore, these calculations were not feasible. Compared to oxygen, the inert gases are minimally susceptible to diffusion limitation. Thus, despite the presence of an oxygen diffusion limitation, the method will continue to estimate V_A/Q distribution. If the measured PO_2 is lower than the predicted PO_2 , then a diffusion limitation is present. The value in the row "DLO2 by Bohr Integration" represents diffusion limitation.

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